Reference: *Biol. Bull.*, **153**: 591–603. (December, 1977)

CONTROL OF ANTENNAL HAIR ERECTION IN MALE MOSQUITOES

H. FREDERIK NIJHOUT[1]

Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014

Thirty years ago Roth (1948) provided conclusive evidence that males of the yellow fever mosquito, *Aedes aegypti*, are sexually attracted to the flight sound of the female. Sound is apparently the only sexual stimulant in this species and males are strongly attracted to and will attempt copulation with any sound source of the appropriate frequency (350–550 Hz). Roth (1948) demonstrated that the male's antennae are the organs by which sound is received. *Aedes aegypti*, like many other species of mosquitoes, has sexually dimorphic antennae. The female antennae are slender and bear rather short setae, whereas those of the male possess 12 equally spaced whorls of very long fibrillae (referred to as *hairs* hereafter), which give the antennae a characteristic bushy appearance. On the basis of extensive experiments, Roth (1948) concluded that the male perceives the female when his long antennal hairs are set into resonant vibration by the sound of the flying female. This vibration is mechanically transmitted to the shaft of the antenna whose motion in turn stimulates the scolopidia of Johnston's organ in the bulbous pedicel of the antennae. Although this phenomenon has only been critically investigated in *Aedes aegypti* (Roth, 1948; Tischner, 1953; Tischner and Schief, 1955; Keppler, 1958; Wishart and Riordan, 1959), a mating response to sound can be demonstrated in males of many other species of mosquitoes, and it is believed that sound is the primary sex attractant in all species whose males possess long antennal hairs (Clements, 1963; Downes, 1969). The mating behavior of species whose males do not possess long antennal hairs, (*e.g., Deinocerites cancer, Opifex fuscus, Culiseta inornata*) suggest that these use pheromones for sexual attraction (Kliewer, Miura, Husbands and Hurst, 1966; Provost and Haeger, 1967).

In *Aedes aegypti* and many other species of the subgenus *Stegomyia*, as well as in *Culex pipiens* and *Toxorhynchites brevipalpis*, the long hairs on the mature male's antenna are permanently erect (Roth, 1948 and personal observations). These species are capable of mating at any time of day. This stands in contrast to the situation in many other species of *Aedes, Anopheles, Culex, Mansonia* and *Psorophora*, in which these hairs are closely appressed to the shaft of the antenna during the daytime and become erect only at dawn and dusk coincident with the brief period of swarming and mating activity (Nielsen and Nielsen, 1958, 1962; Nielsen, 1964; Foster and Lea, 1975). In addition, males of species like *An. balabacensis, An. maculatus* and *Ae. vexans* that will not mate under laboratory conditions are not known to erect their antennal hairs in captivity (Dr. George B. Craig, Jr., University of Notre Dame, personal communication; and personal observations).

[1] Present address: Department of Zoology, Duke University, Durham, North Carolina 27706.

Young adult males of *Aedes aegypti*, whose antennal hairs have not yet erected, do not respond to the female flight sound. This suggests that males are incapable of hearing the females when their antennal hairs are recumbent on the shaft of the antenna (Roth, 1948; Wishart and Riordan, 1959). It is therefore reasonable to assume that males of those species which show a daily rhythm of antennal hair erection are not capable of detecting females (and thus incapable of mating) during the daytime when their antennal hairs are recumbent.

The present paper deals with the control of the mechanism that induces antennal hair erection in *Anopheles stephensi* and demonstrates that antennal hair erection is under direct nervous control, disproving the common notion (*e.g.*, Downes, 1969) that changes in blood pressure are the causative event.

Materials and Methods

Mosquitoes were reared in a temperature-controlled insectary at 27° C under a 16L:8D photoperiod regime. Adults had continuous access to a dilute sugar solution. All experiments were performed on 4–9 day old males at a room temperature of 23–25° C during a six hour period in the middle of the light phase of the photoperiod.

The saline used in these experiments was that of Lum (1961) adjusted to pH 6.5. All drugs were purchases from SIGMA Chemical Co. with the exception of Phentolamine (gift from Ciba-Geigy Corp.), Terbutaline (gift from Astra Pharmaceutical Products, Inc.), and Salbutamol (gift from Schering Corp.). Experiments on intact males were performed with the animals restrained on their backs on a glass slide covered with a thin layer of petroleum jelly. Injections were done *via* a glass capillary drawn to a fine tip on a micropipette puller. About 0.10–0.15 μl of the experimental solution was injected into the thorax of each male. Isolated antennae were prepared by grasping the male's proboscis with forceps and cutting just below the pedicel of the antennae with iridectomy scissors. In this way

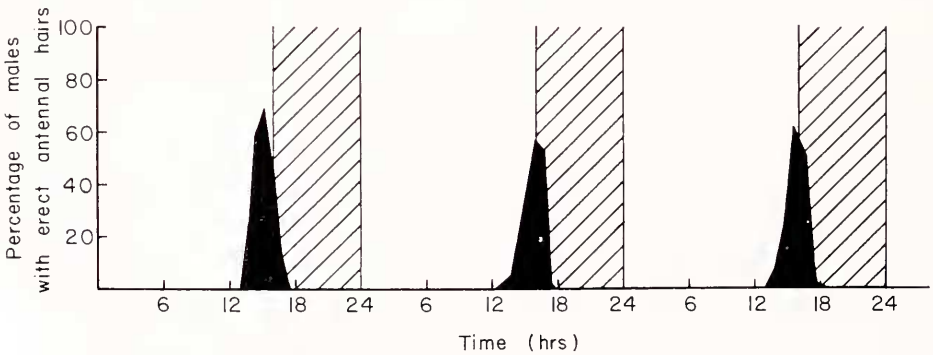

Figure 1. Rhythm of antennal hair erection in males of *Anopheles stephensi* under a 16L:8D photoperiod with abrupt light-dark transitions. Shaded areas represent periods of darkness. Data represent the percentage of males with erect antennae during three consecutive photoperiodic cycles in a population of 300 males held in gallon-size cages. Males begin to erect their antennal hairs about 3 hours before lights-off. All males have recumbent hair again 6 hours later.

antennae, palps and proboscis came off as a unit, sometimes with a small portion of compound eye attached. The proboscis and palps thus served as a convenient handle to transfer antennal sets without damage to the delicate antennae. Control experiments showed that the presence of proboscis, palps and the pedicel of the antenna in no way affected the response of isolated antennae. Antennal sets were floated, base down, on a drop of saline resting on a siliconized glass slide. This slide was placed in a petri dish lined with moist filter paper to prevent dessication of the preparations. In the absence of further stimulation the antennal hairs remained recumbent for at least four hours in such preparations. A scoring system was developed for assaying the effectiveness of various chemical stimuli in inducing antennal hair erection. An antenna with all hairs recumbent was given a score of 0, while an antennae with all hairs erect received a score of 10. Intermediate scores (1–9) were obtained by estimating the proportion of hair whorls that had been erected (see Figure 3). When the mean score of 10–20 antennal sets was taken, the response to a given drug solution was found to be quite reproducible. All experiments reported below were performed with males of *Anopheles stephensi*.

Results

The antennal hair erection rhythm of *Anopheles stephensi* males was studied under a 16L:8D photoperiod with abrupt day-night transitions. Animals used in this experiment were maintained in gallon-size cages in the absence of females. Figure 1 shows that under these conditions males began to erect their antennal hairs in anticipation of the onset of darkness, and the percentage of males with erect antennal hairs was already on the decline at lights-off. This behavior indicates that the hair erection rhythm is governed by a circadian clock and is not merely a response to darkness. In fact, in *An. stephensi*, antennal hair erection *cannot* be induced at any time during the photophase either by sudden darkness or by gradual light dimming. Under the conditions of this experiment, there was no hair erection at or about the lights-on signal. Thus, antennal hairs were recumbent at all times except for a 3–4 hour period immediately preceding the onset of darkness. A maximum of 60% of the males in the experimental population had erect hairs at any one time. It is not known at present whether this reflects an asynchrony among members of the population or whether only 60% of the individuals are capable of responding during any one cycle. Preliminary evidence indicates that the presence of females does not affect the hair erection rhythm of the males.

In larger cages (8 ft^3) it was possible to find a few males (less than 1% of the population) with erect antennal hairs at any time of day. These males actively copulated with flying females. This stands in contrast to the situation in gallon-size cages where antennal hair erection *and* mating were sharply restricted to the period indicated in Figure 1.

Response of intact males to injected drugs

Possible nervous involvement in the control of antennal hair erection was investigated by injecting a number of known and putative neurotransmitters and other pharmacological agents into intact males. Rapid erection of antennal hairs occurred upon injection of various catecholamines and other sympathomimetic agents. The lowest concentration of these substances that caused erection of all hairs within five

minutes and the persistence of erection for at least twenty minutes were: DL-synephrine, 0.1 mM; L-epinephrine, 0.2 mM; DL-octopamine, 1 mM; L-phenylephrine, 2 mM; DL-norepinephrine, 4 mM; and dopamine, 8 mM. The rather high concentrations required are probably due to rapid metabolism of the drugs making it unlikely that differences in activity of the various isomers could be detected. Injections of up to 30 mM acetylcholine, atropine, physostigmine, neostigmine, pilocarpine, γ-aminobutyric acid or 5-hydroxytryptamine were without effect. Injection of all sympathomimetics induced simultaneous erection of all hair whorls 2–3 min after injection except for phenylephrine which first induced erection of the proximal whorls followed progressively by the more distal ones over a 3 minute period. The former response can be explained on the basis of a rapid distribution of the drugs throughout the antenna by the circulatory system and compares favorably with the normal (photoperiodically stimulated) erection pattern in which all hairs became erect simultaneously in the course of about 5 min. The response to phenylephrine is not readily interpreted at present. Injections of synephrine, epinephrine and occasionally octopamine also caused flexion of the distal palp segments identical to that observed during spontaneous antennal hair erection at dusk (Figure 2a, b). Flexed palps are characteristic of swarming males, but no function has yet been elucidated for this condition. Finally, intact males also erected their antennal hairs upon anesthesia with either chloroform or CO_2. Depth of the anesthesia, judged by the time required for recovery, determined the degree to which the hairs were erected, as well as the duration of erection.

Response of isolated antennae to sympathomimetic drugs

The results presented above indicate that blood-borne sympathomimetic agents can induce antennal hair erection. To determine whether these compounds acted

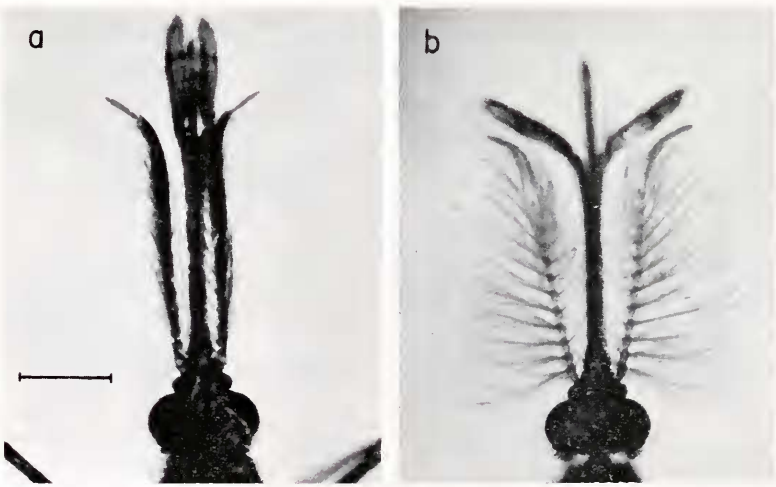

FIGURE 2. a. Head of male *Anopheles stephensi* with recumbent antennal hairs. This is the characteristic configuration during most of the day; and b, male with erect antennal hairs and flexed palp tips about one hour prior to the onset of darkness. Only males with erect antennal hairs can detect a female. Bar is 0.5 mm.

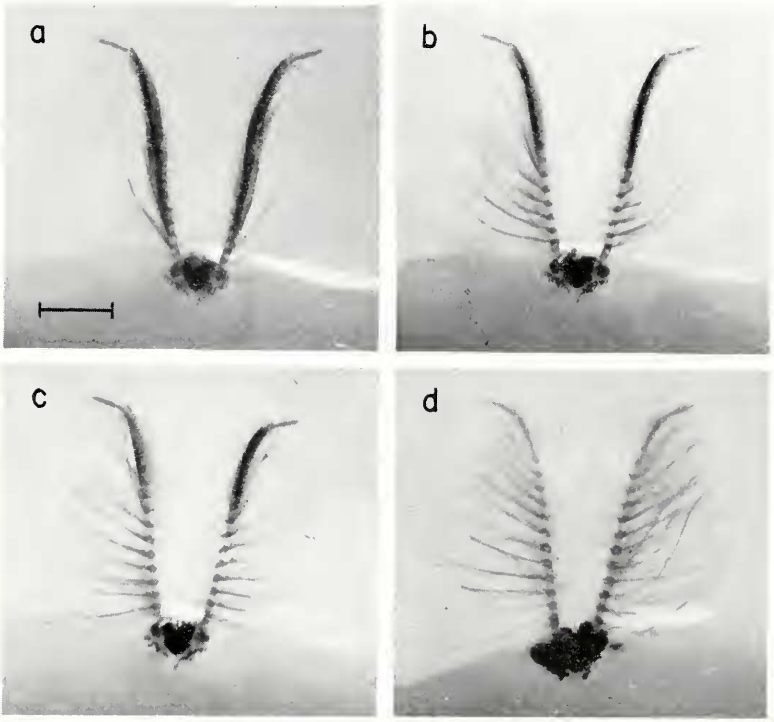

FIGURE 3. Sequence of hair erection in isolated antennae floating on a drop of saline containing 0.5 mM synephrine: a, one minute after initiation of experiment; b, three minutes; c, five minutes; and d, twelve minutes. The response of these antennae is scored as 0, 4, 6 and 10, respectively, according to the scoring system described in the text. Bar is 0.5 mm.

directly on the effectors in the antennae, isolated antennae were floated, base-down, on drops of saline containing appropriate concentrations (1–10 mM) of a sympathomimetic agent. Under these conditions, the proximal hairs became erect in about 2 to 3 minutes and were gradually followed by the more distal hairs (Figure 3). It is likely that this sequence of hair erection followed the diffusion of the exogenous drug up the antennal shaft. These results show that blood-borne sympathomimetics act directly on the effectors for hair erection in the antennae. Flexion of the distal palp segments never occurred in these isolated preparations. This suggests that the palps responded to a secondary stimulus (possibly arising in the central nervous system) and not to the drug itself.

Since isolated antennae can erect their hairs normally it is clear that changes in blood pressure are not involved in this response. Isolated antennae provided a convenient and reliable assay for studying the effects of various stimuli on antennal hair erection. The response was quantified by determining the proportion of hair whorls on an antenna that had become erect at various times after exposure to the drug solution (see Methods and Fig. 3). Figure 4 illustrates the time course of the response of isolated antennae to various concentrations of synephrine, epinephrine and octopamine. The point at which each curve reached a plateau presumably

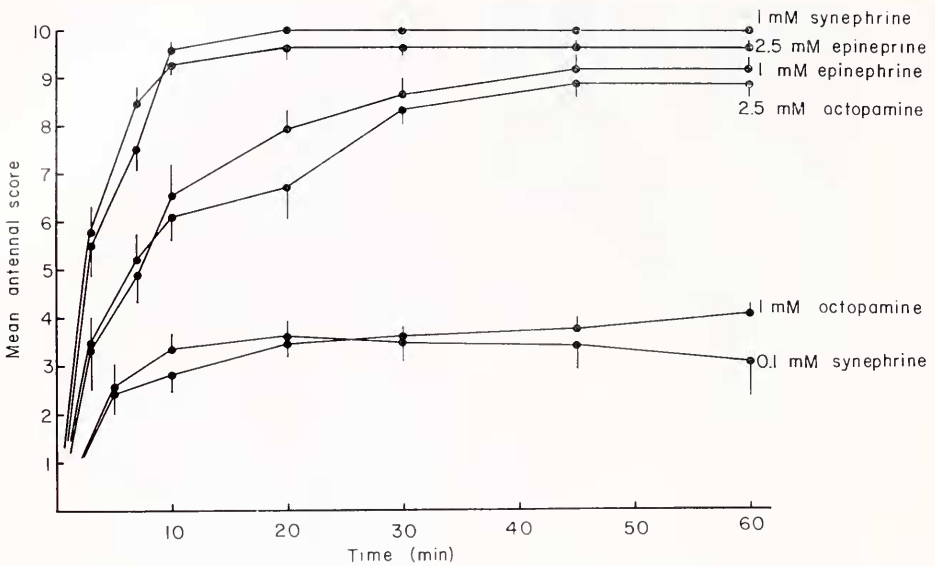

FIGURE 4. Response of isolated antennae to various sympathomimetic agents. Each curve represents the mean of two replicates of 24 antennae each. Scoring system for hair erection reflects the proportion of hair whorls that had become erect at a given time (*cf.*, Figure 3). Bars indicate standard errors.

reflects the achievement of a suprathreshold concentration of the amine throughout the antennae. Some antennae were slightly damaged in setting up the experiment and failed to erect all hairs. This accounts for plateaus somewhat below the maximum mean score. More significantly, the curves for 0.1 mM synephrine and 1 mM octopamine reached a plateau when only the proximal 30% of the hairs had become erect. This stands in contrast to the findings on intact animals where these concentrations of drugs induced erection of *all* antennal hairs. In the latter case, swift metabolism of the drugs was evident by the short persistence of hair erection (20–30 minutes). If metabolism of these drugs also occurred in isolated antennae, then the failure of the more distal hairs to become erect was probably due to the fact that a suprathreshold concentration could not be achieved beyond a point along the antennal shaft where metabolism of the drug balanced its arrival by diffusion. This point shifts progressively more distally with increasing concentrations of drug.

Effect of picrotoxin

Picrotoxin is a central nervous system stimulant that acts by blocking the activity of γ-aminobutyric acid, an inhibitory neurotransmitter in vertebrates and invertebrates. Injections of 1 mM picrotoxin into intact males induced rapid erection of all antennal hairs that persisted for several hours (threshold dose, as defined above, was 0.1 mM). In contrast to the action of sympathomimetic agents, picrotoxin did not induce hair erection in isolated antennae even at a 30 mM concentration. In order to localize the site of action of picrotoxin, progressively larger portions of head and thorax were removed with the antennae and floated with the cut surface onto a

saline drop containing 1 mM picrotoxin. Figure 5 shows that the presence of the head and the anterior half of the thorax were required for picrotoxin to stimulate hair erection. It is unlikely that the inactivity of picrotoxin in isolated antennae was due to a failure of this substance to penetrate the antennae, because fluorescence microscopy showed that fluorescein, added to the picrotoxin-saline, readily diffused up the antennal shaft. Conversely, when isolated head-thorax preparations were treated with a picrotoxin solution that also contained fluorescein, no dye could be detected in the antennae for at least 20 minutes after the hairs became erect. This observation indicated that picrotoxin probably had not penetrated the antennae at the time that the hairs became erect.

These results suggest that picrotoxin acted on a center in the thorax that, in turn, was responsible for inducing erection of the antennal hairs. When males that had been injected with picrotoxin were decapitated, the antennal hairs on the isolated heads assumed their recumbent position within 3 min, while on intact control animals, they remained erect for several more hours. Thus, the thoracic center is also required for the maintenance of hair erection. Destruction or bisection of the brain completely abolished the response to picrotoxin in otherwise intact animals. This observation indicated that the brain is needed in this response, although its precise role and the nature of its interaction with the thoracic center are not clear at present.

Pathway of control for the erectile mechanism

There are two ways in which the thoracic center that is activated by picrotoxin could control the erection of antennal hairs. It may cause the release of a humoral substance from a site outside the antennae, which in turn is carried to the effectors by the bloodstream. Alternatively, it may cause a nervous signal to be sent directly

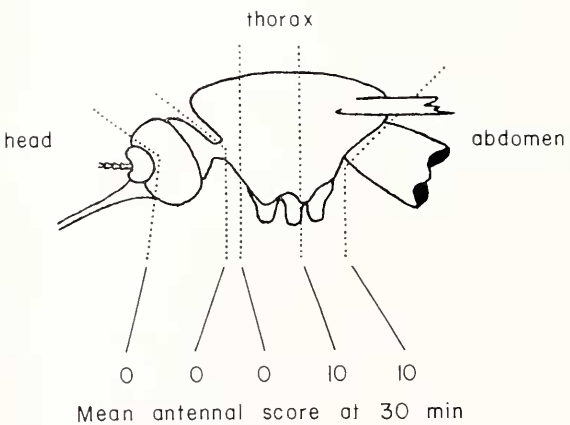

FIGURE 5. Response of various sections of male mosquitoes to picrotoxin. Cuts were made at the level of the dotted lines and the anterior portions floated with their cut ends on a drop of saline containing 1 mM picrotoxin. Numbers represent the degree to which antennal hairs had erected 30 minutes after exposure to picrotoxin. Each operation was replicated 35–40 times. It appears that the head as well as a portion of the thorax are required in the response to picrotoxin.

to the effectors in the antennae. To distinguish between these alternatives nervous input into the right-hand antenna was abolished in 60 animals by cutting the antennal nerve with a hooked needle through an aperture made by cutting off the proboscis and palps at their base. In 51 of these animals only the hairs of the left-hand antenna became erect upon subsequent injection of a solution of picrotoxin (1 mg/ml) containing a small amount of fluorescein. In two animals a few of the more distal hair whorls became erect, and all hairs of the right-hand antenna erected in the remaining seven. Upon dissection, it appeared that the antennal nerve of the latter animals was not significantly damaged. In order to ensure that the operation had not interfered with the circulation of hemolymph into the right-hand antennae, all antennal pairs were examined under a fluorescence microscope about five minutes after the hairs on the left-hand antenna had fully erected. The distribution of fluorescein in both antennae appeared identical in most cases. In several instances the antenna on the operated side contained more dye than the one on the intact side. In addition, twelve males that had failed to erect the hairs on their right-hand antennae were injected with a solution of synephrine (0.03 μl, 1 mM) five to ten minutes after the hair on the left-hand antenna had become erect. The synephrine injection induced erection of all hairs on the right-hand antenna within two minutes. It is clear from these experiments that circulation into the antenna on the operated side was not significantly affected, nor was the erectile mechanism of the antenna organically damaged. The results of these experiments indicate that the erection of antennal hairs is not controlled by a blood-borne factor. The most likely alternative is that hairs are controlled by direct nervous input into the antennae.

Electrical stimulation of antennae

Further evidence for nervous control of the erectile mechanism of antennal hairs was provided by experiments in which antennae, *in situ* or isolated, were electrically stimulated. Square wave stimuli of 150–200 mV amplitude and 10 msec duration at a frequency of 20 cps were applied to intact animals by inserting one electrode into the thorax and the other into the cut end of one antenna. Antennal hairs became erect within 30 seconds of stimulation. Erection persisted as long as stimulation was applied and subsided about one minute after cessation of stimulation. Hairs on the unstimulated antenna of these experimental animals never showed any evidence of erection. Electrical stimulation also induced hair erection in isolated antennae. In these experiments single antennae were arched between two drops of saline resting on a siliconized microscope slide so that only the two ends of the antenna were immersed. The stimulating electrodes were then inserted into these saline drops. Hair erection followed shortly upon initiation of stimulation though somewhat higher voltages (300–450 mV) were required to evoke a response in these isolated antennae than were necessary in antennae *in situ*. This difference in apparent sensitivity was probably due to a greater separation of electrodes in the latter experiment.

Evidence for the involvement of antennal motor neurones in the response to electrical stimulation was obtained from two different experiments. In the first set, males were injected with tetrodotoxin (1 μg/μl in saline), a substance that specifically blocks the sodium current in axons, rendering them incapable of conducting action potentials. Four to six hours later their antennae (*in situ* or isolated) were

electrically stimulated as described above. Only one instance of hair erection was found in ten animals treated this way, and in that single instance the antennal hairs became only partially erect. When isolated antennae of these tetrodotoxin-treated males were subsequently floated on drops of saline containing 5 mM octopamine, all hairs erected normally. Thus, tetrodotoxin did not affect the effector mechanism itself but probably prevented response of motor axons in the antenna.

In the second set of experiments, isolated antennae were floated on drops of saline containing an elevated concentration of potassium ions (60 mM ; isotonicity of the saline was conserved by a proportional decrease in the concentration of NaCl). Such high concentrations of potassium are known to cause depolarization of axons and release of neurohormones or neurotransmitters in insects (Maddrell and Gee, 1974; Usherwood, 1974). Antennal hairs began to erect within one minute after exposure to high-potassium saline, and all hairs were fully erect ten minutes later. The hairs remained erect for 20–30 minutes and then gradually returned to their recumbent position. All hairs were fully recumbent by 1.5–2 hours later. After all the hairs had become recumbent, the antennae were electrically stimulated as described above. Not a single one of 12 antennae tested in this way erected its hairs. However, all of these antennae erected their hairs normally when they were subsequently placed on saline drops containing 5 mM octopamine. It is thus clear that the effector mechanism for hair erection was not affected by the high-potassium treatment. It seems reasonable to conclude from these results that the loss of response in antennae exposed to high-potassium saline was due to local depletion of neurotransmitter at the motor axon-effector synapse and possibly also due to the fact that action potentials could no longer be generated in the chronically depolarized axons.

Attempts to demonstrate Ca^{++}-dependence of antennal hair erection have failed so far, probably due to the fact that there are numerous hemolymph cavities within the antenna that are not readily flushed out [see Risler (1953) for a description of the anatomy of a mosquito antenna] and could serve to sequester calcium ions.

Evidence for second messenger involvement in the response to sympathomimetics

There is increasing, though still circumstantial, evidence that catecholamines and other sympathomimetic agents induce the synthesis of cyclic adenosine monophosphate (cAMP) in their target cells and that the increased levels of cAMP mediate the cell-specific reactions to these substances. In order to determine a possible role of cyclic nucleotides in mediating antennal hair erection, isolated antennae were incubated on saline drops containing cAMP or one of its derivatives or cGMP, in the presence or absence of theophylline. Figure 6 illustrates the results of these experiments and shows that, in the presence of theophylline, 8-bromo-cAMP was highly effective in stimulating hair erection, whereas this compound was inactive when supplied in the absence of theophylline. Theophylline alone produced an intermediate response. Cyclic AMP and dibutyryl-cAMP did not enhance the response to theophylline. Presumably these compounds were unable to penetrate the effector cell membrane.

The response to 3mM cyclic GMP in the presence of theophylline was identical to the response to 8-bromo-cAMP. Cyclic GMP did not evoke antennal hair erection in the absence of theophylline.

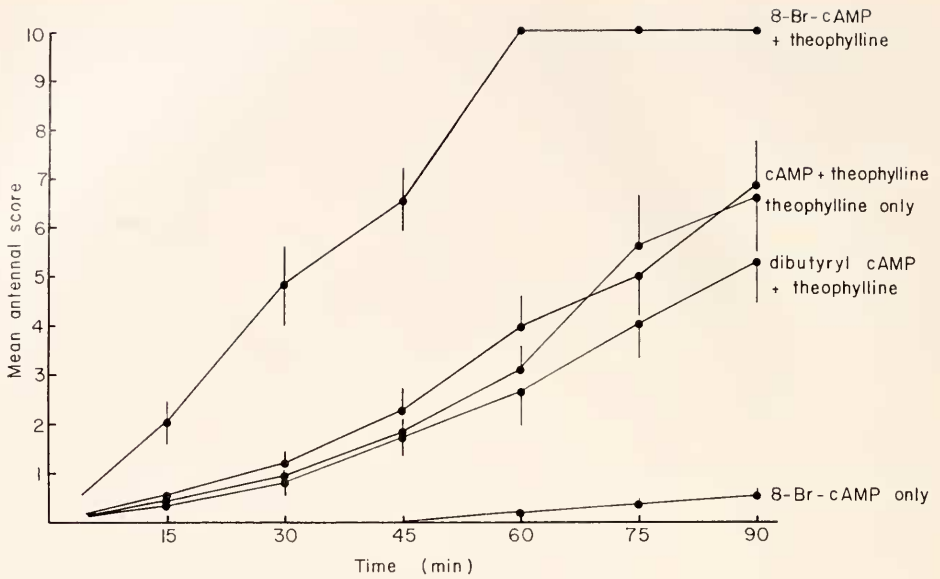

FIGURE 6. Effect of cyclic nucleotides and theophylline on hair erection in isolated antennae. Cyclic nucleotide concentrations were 3 mM; theophylline, 8 mM throughout. Each curve represents the mean of 6 replicates and a total of 90–124 antennae. Bars indicate standard errors.

The experiments described above show that cyclic nucleotides can mimic the action of sympathomimetic agents. Although a role for cyclic nucleotides in mediating the action of the neurotransmitter is indicated, further proof of this will have to await determination of a rise in their titer after stimulation.

Nature of the adrenergic receptor

In view of the specificity of hair erection for exogenous sympathomimetic agents, it was of some interest to determine the nature of the cell surface receptor. Groups of males were pretreated with an injection of 10 mM phentolamine (a specific α-adrenergic blocking agent) or 10 mM propranolol (a β-adrenergic blocking agent). After 45 minutes, antennal sets were removed and floated on saline drops containing either 1 mM epinephrine or 1.5 mM octopamine with 10 mM of one or the other blocking agent. Inhibition of the response to octopamine and epinephrine occurred in the presence of phentolamine, but propranolol had not effect whatsoever (Table I). These results show that the cell receptor (presumably on the effector cells for antennal hair erection) resembles the vertebrate α-adrenergic receptor. The specificity of the receptor was examined further by injecting intact males or treating isolated antennae with terbutaline or salbutamol, sympathomimetic agents having exclusively β-adrenergic activity. These compounds were totally ineffective in inducing antennal hair erection in concentrations up to 30 mM. When phentolamine-blocked isolated antennae were treated with 8-bromo-cAMP plus theophylline, antennal hair erection occurred normally showing blockage was not due to a non-specific toxic effect of this drug (Table I).

Discussion

The evidence presented above strongly suggests that erection of the antennal hairs of males of *Anopheles stephensi* is under direct nervous control. The finding that partial severance of the antennal nerve can abolish erection of the proximal hairs, while leaving the distal ones under central control, indicates that motor neurons must extend up the entire length of the antennal flagellum. This is a surprising finding since motor neurons have been assumed to be absent from antennae of pterygote insects.

Knowledge of the internal anatomy of mosquito antennae is limited to the work of Risler (1953, 1955), whose findings and descriptions offer no clue as to the effector organ of hair erection. A few cells occur at the base of each antennal hair. These were believed to be sensory neurons (Risler, 1953), but recent evidence indi-

Table I

Effects of sympathomimetic drugs and adrenergic blocking agents on hair erection in isolated antennae of Anopheles stephensi *males.*

Compound	Conc (mM)	Agonistic activity	Blocking activity	Mean score at 60 min*
Effect of adrenergic blocking agents				
Epinephrine	1	α, β	—	9.8
Octopamine	1.5	α, ?	—	5.8
Epinephrine plus Propranolol	1 10	α, β —	— β	9.1
Octopamine plus Propranolol	1.5 10	α, ? —	— β	6.0
Epinephrine plus Phentolamine	1 10	α, β —	— α	0.0
Octopamine plus Phentolamine	1.5 10	α, ? —	— α	0.6
Specific α- and β-adrenergic drugs				
Synephrine	5	α	—	10
Phenylephrine	10	α	—	10
Norepinephrine	10	α	—	9.8
Dopamine	10	α	—	5.8
Terbutaline	30	β	—	0.0
Salbutamol	30	β	—	0.0
α-blockage bypass with cyclic nucleotide				
Phentolamine	10	—	α	
8-bromo-cAMP	8	—	—	9.2
Theophylline	10	—	—	

* Each score is the mean response of 3 replicates of 18–24 antennae each.

cates that at least some of these cells are part of the effector mechanism for antennal hair erection (Nijhout and Sheffield, in preparation). Definitive proof of synaptic transmission at the effector site will probably prove exceedingly difficult, if not impossible, to obtain. The antennae of this species are only 20 μm in diameter and direct access to the antennal nerve is hindered by the fact that it is encased in two layers of the antennal skeleton.

The only pharmacological agents capable of eliciting hair erection in isolated antennae are sympathomimetic agents and, of these, only those that possess α-adrenergic activity. Since the response (of isolated antennae) to these drugs is blocked by phentolamine but not by propranolol (Table I), it appears that an α-adrenergic-like receptor occurs in the membrane of the effector cells. It is therefore possible that a catecholamine or a phenolic amine is the natural neurotransmitter that acts on the effector cells in the antennae. The relative potency of the sympathomimetics used is identical to that found in the firefly lantern (Carlson, 1968). Robertson and Carlson (1976) have suggested that octopamine is the natural neurotransmitter in that system. However, other catecholamines, particularly dopamine, have been implicated as neurotransmitters in nonmuscular effector organs of insects such as the salivary glands (Bland, House, Ginsberg and Lazlo, 1973; Robertson, 1975). Although dopamine has a relatively low activity in induction of hair erection, its possible involvement in this process cannot be ruled out at present. It should be noted here that it has proven impossible to demonstrate the presence of catecholamines in antennae by means of the Falck-Hillarp catecholamine-fluorescence technique due to high background fluorescence in these organs. Thus, final resolution of the identity of the neurotransmitter will be possible only after detailed biochemical studies.

The results of experiments using picrotoxin indicate that the nervous system in the head is not sufficient to either initiate or maintain erection of the antennal hairs. Rather, a portion of the thoracic nervous system is required for picrotoxin-stimulated hair erection. This is a puzzling finding, since it would be reasonable to assume exclusive cerebral control over this event. The data presented above do not allow any conclusions about the relative roles of the cerebral and thoracic nervous systems in hair erection. Any further speculation on the central control of hair erection will have to await more detailed neurophysiological investigations.

I wish to thank Drs. Louis H. Miller and Robert W. Gwadz for critical reading of the manuscript.

Summary

The long fibrillae, or hairs, on the antennae of male mosquitoes are essential for the detection of the female flight sound, the sole sexual attractant. In *Anopheles stephensi*, as in many other genera and species, these long hairs are recumbent on the shaft of antenna during the daytime and become briefly erect at dusk, coincident with swarming and mating activity. Evidence is presented that erection of the antennal hairs is under direct nervous control. Isolated antennae can be induced to erect their hairs only in the presence of α-adrenergic agonists, and this response is blocked in the presence of the α-adrenergic blocking drug, phentolamine. Thus, a cell surface receptor, resembling the vertebrate α-adrenergic receptor, probably

mediates the response to the natural neurotransmitter. The action of α-adrenergic drugs is mimicked by cyclic nucleotides and also by theophylline.

LITERATURE CITED

Bland, K. P., C. R. House, B. L. Ginsberg, and I. Lazlo, 1973. Catecholamine transmitter for salivary secretion in the cockroach. *Nature, New Biol.*, **244**: 26–27.

Carlson, A. D., 1968. Effect of drugs on luminescence in larval fireflies. *J. Exp. Biol.*, **49**: 195–199.

Clements, A. N., 1963. *The physiology of mosquitoes.* Macmillan, New York.

Downes, J. A., 1969. The swarming and mating flight of diptera. *Ann. Rev. Entomol.*, **14**: 271–298.

Foster, W. A., and A. O. Lea, 1975. Sexual behavior maturation in male *Aedes triseriatus* (Diptera: Culicidae): a re-examination. *J. Med. Entomol.*, **12**: 459–463.

Keppler, E., 1958. Über das Richtungshören von Stechmücken. *Z. Naturforsch.*, **138**: 280–284.

Kliewer, J. W., T. Miura, R. C. Husbands, and C. H. Hurst, 1966. Sex pheromones and mating behavior of *Culiseta inornata* (Diptera: Culicidae). *Ann. Entomol. Soc. Am.*, **59**: 530–533.

Lum, P. T. M., 1961. The reproductive system of some Florida mosquitoes. I. The male reproductive tract. *Ann. Entomol. Soc. Am.*, **54**: 397–401.

Maddrell, S. H. P., and J. D. Gee, 1974. Potassium-induced release of the diuretic hormones of *Rhodnius prolixus* and *Glossina austeni: Ca* dependence, time course and localization of neurohaemal areas. *J. Exp. Biol.*, 61: 155–171.

Nielsen, E. T., and H. T. Nielsen, 1958. Observations on mosquitoes in Iraq. *Entomol. Medd.*, **28**: 282–321.

Nielsen, H. T., 1964. Swarming and some other habits of *Mansonia perturbans* and *Psorophora ferox* (Diptera: Culicidae). *Behaviour*, **20**: 67–89.

Nielsen, H. T., and E. T. Nielsen, 1962. Swarming of mosquitoes. Laboratory experiments under controlled conditions. *Entomol. Exp. Appl.*, **5**: 14–32.

Provost, M. W., and J. S. Haeger, 1967. Mating and pupal attendance in *Deinocerites cancer* and comparison with *Opifex fuscus* (Diptera: Culicidae). *Ann. Entomol. Soc. Am.*, **60**: 565–574.

Risler, H., 1953. Das Gehörorgan der Männchen von *Anopheles stephensi* Liston (Culicidae). *Zool. Jahrb. Abt. Anat. Ontog. Tiere*, **73**: 165–178.

Risler, H., 1955. Das Gëhororgan der Männchen von *Culex pipiens* L., *Aëdes aegypti* L. und *Anopheles stephensi* Liston (Culicidae), eine vergleichend morphologische Untersuchung. *Zool. Jahrb. Abt. Anat. Ontog. Tiere*, **74**: 478–490.

Robertson, H. A., 1975. The innervation of the salivary gland of the moth, *Manduca sexta:* evidence that dopamine is the transmitter. *J. Exp. Biol.*, **63**: 413–419.

Robertson, H. A., and A. D. Carlson, 1976. Octopamine: presence in firefly lantern suggests a transmitter role. *J. Exp. Zool.*, **195**: 159–164.

Roth, L. M., 1948. A study of mosquito behavior. An experimental laboratory study of the sexual behavior of *Aedes aegypti* (Linnaeus). *Am. Mid. Nat.*, **40**: 265–352.

Tischner, H., 1953. Über den Gehörsinn von Stechmücken. *Acustica*, **3**: 335–343.

Tischner, H., and A. Schief, 1955. Flüggerausch und Schallwahrnehmung bei *Aedes aegypti* L. (Culicidae). *Zool. Anz. (Suppl.)*, **18**: 453–460.

Usherwood, P. N. R., 1974. Nerve-muscle transmission. Page 245–305 in J. E. Treherne, Ed., *Insect neurobiology.* North-Holland Publ. Co., Amsterdam.

Wishart, G., and D. F. Riordan, 1959. Flight responses to various sounds by adult males of *Aedes aegypti* (L.) (Diptera; Culicidae). *Can. Entomol.*, **91**: 181–191.